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NUTRITION REVIEWS

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NUTRITION REVIEWS

VOL. 11 APRIL 1953 No. 4

THE NATURE OF X-DISEASE IN CATTLE

In 1947, a new disease in cattle of unknown etiology was designated X-disease by P. Olafson (*Cornell Veterinarian* **37,** *279 (1947)*). This disease is also often referred to as hyperkeratosis. Although X-disease seems to be more common in some sections of the country than in others, it has been recognized in nearly every state in the Union. It has been estimated that cattle worth $4,000,000 have died or have had to be slaughtered because of this disease in the Southeastern states alone.

The disease, according to veterinarians, is most common in growing animals 3 to 12 months of age. The affected animals develop a thickened crusty skin, first on the sides of the neck, back and rump, and then extending down the sides. Excessive lacrimation is a common finding. Changes often occur in the mouth, causing wart-like growths, soreness, and difficulty in eating. The animals often lose weight very rapidly, and diarrhea and polyuria are usually present.

The disease has been under careful study since 1950 through the cooperative efforts of sixteen State Agricultural Experiment Stations, the Bureau of Plant Industry, Soils, and Agricultural Engineering, and the Bureau of Animal Industry of the U. S. Department of Agriculture.

Possible causative agents came under careful scrutiny when the disease was produced successfully in 1951 in four different laboratories (*Report of the Chief of the Bureau of Animal Industry, U. S. Department of Agriculture, p. 37 (1951)*). At Pennsylvania State College, X-disease was produced experimentally in calves by feeding hay from a farm where there had been two serious outbreaks of the disease following feeding of this hay. The disease was produced at Ithaca, New York, by feeding calves a processed feed concentrate obtained from the State Veterinarian of Vermont (P. Olafson and K. McEntee, *Cornell Veterinarian* **41,** *107 (1951)*). The particular feed, a processed wheat concentrate, had been suspected of causing hyperkeratosis.

Workers at the Indiana Experiment Station were able to produce X-disease experimentally in calves on a farm where field cases of the disease had occurred earlier (F. V. Washko, J. W. Green, and C. R. Donham (*Cornell Veterinarian* **41,** *346 (1951)*). No animals had been on the farm for four months before the experimental animals were brought in and the latter developed the disease on pasture.

At the Nebraska Experiment Station, the disease occurred unexpectedly in a feeding trial with calves. Of 150 animals in the experiment, 130 developed the disease (C. Olson, R. H. Cook, and E. M. Brouse, *Am. J. Vet. Res.* **11,** *355 (1950)*). The feed, a pelleted dehydrated alfalfa and dicalcium phosphate, was later shown to be responsible for the disease (Olson and Cook, *Ibid.* **12,** *261 (1951)*).

These observations, made independently in various sections of the United States, were suggestive that the field outbreaks of the disease might be caused by toxic materials in the feed.

The workers at Cornell University proceeded with a fractionation of an ether extract prepared from the processed concentrate and reported that the causative agent was present in the free fatty acid fraction (K. McEntee, W. Hansel, and P. Olafson (*Cornell Veterinarian* **41,** *237 (1951)*), and that the ether extract residue and the phenolic and neutral steroid fractions of the ether extract did not produce the disease. Later work showed that the toxic material itself was not a fatty acid (Hansel, McEntee, and Olafson, *Ibid.* **41,** *367 (1951)*).

During the investigation of an outbreak

of X-disease in Virginia, W. B. Bell (*Virginia J. Sci.* **3,** *71 (1952)*) noted that a toxic substance or substances present in a lubricant might be responsible for the disease. The disease was produced by feeding the lubricant to calves; as little as 70 g. administered by mouth over a period of seventeen days produced typical symptoms and lesions. Cooperative work was initiated with the manufacturer of the lubricant and the harmful agent was identified as a highly chlorinated naphthalene (Bell, *Ibid.* **3,** *169 (1952)*). A 3 per cent solution of the highly chlorinated naphthalene in mineral oil, administered at a rate of 15 g. over a period of seven days, produced symptoms of X-disease. The original lubricant minus the chlorinated naphthalene failed to produce the disease. Bell points out that the cooperating petroleum manufacturer has ceased to use the highly chlorinated naphthalene as a chemical additive in the chassis lubricant that was originally implicated in X-disease.

Workers at the Tennessee Agricultural Experiment Station (D. Sikes and M. E. Bridges, *Science* **116,** *506 (1952)*; Sikes, J. C. Wise, and Bridges, *J. Am. Vet. Med. Assn.* **121,** *337 (1952)*) demonstrated that typical symptoms of X-disease could be produced in cattle by the feeding of pentachloronaphthalene, octachloronaphthalene, a chassis lubricant, or a used tractor crankcase oil. Two g. of pentachloronaphthalene fed daily for forty days produced typical symptoms in 500-pound calves.

Recently, the Cornell group (P. Olafson, K. McEntee, and W. Hansel, *Proceedings of the 1952 Cornell Nutrition Conference for Feed Manufacturers, Nov. 6 and 7, p. 72 (1952)*) has reported the fractionation of an X-disease-producing crystalline material from a wood preservative which had been implicated as a causative agent in X-disease in Germany (K. Wagener, *J. Am. Vet. Med. Assn.* **119,** *133 (1951)*; **120,** *145 (1952)*). The crystalline material was nearly identical in elemental composition with trichloronaphthalene, although the authors state that more highly chlorinated naphthalenes were probably also present. Ultraviolet and infrared absorption curves of commercial chlorinated naphthalenes and the active material were practically identical. A second active fraction obtained from the same wood preservative, a green oily material, was not positively identified, but was reported to possess an odor and a boiling point much like trichlorobenzene.

Both the Cornell and the Tennessee studies have indicated that the causative agent in experimental X-disease is transmitted through cow's milk. Calves nursed on cows receiving the disease-producing agent developed typical symptoms of X-disease. The harmful agents are apparently likewise absorbed through the skin. The used crankcase oil in the Tennessee study produced the disease in both the cow and her suckling calf when the oil was applied on the skin along the back of the cow. So far as the chlorinated compounds are concerned, the Tennessee results indicated that the greater the degree of chlorination the greater the toxicity.

According to Sikes and associates (*loc. cit.*), the chlorinated naphthalenes have many uses in industry. They are used as insecticides, or as electrical insulators for wires and coils, and they may be used as additives to lubricants, crankcase oils, or cutting oils. These workers point out that under modern agricultural practices, cattle may have a number of sources of these compounds; for example, crankcase oil is used widely as an insecticide on cattle.

The various investigators are in general agreement that these recent findings should not be construed as indicative that all cases of X-disease are traceable to chlorinated naphthalenes. The announcement by the U. S. Department of Agriculture, implicating chlorinated naphthalene compounds, stated that such compounds are found in various products used on the farm, but would not normally be found in the feeds which

have produced X-disease (*J. Am. Vet. Med. Assn.* **121,** *194 (1952)*).

Olafson and associates in their most recent paper (*loc. cit.*) point out that there are indications that the active fraction of the processed wheat concentrate is not a highly chlorinated naphthalene. The Tennessee studies indicate that hyperkeratosis, as diagnosed by some veterinarians, may be two different diseases. One of these is that which is readily produced experimentally, while the other has comparable symptoms only in its early stages.

Efforts to demonstrate an infectious agent have failed in studies at the Texas and Georgia Experiment Stations, according to the 1951 report of the Bureau of Animal Industry.

Excess molybdenum has been implicated as a causative agent (*Proceedings of the Johns Hopkins University Conference on X-disease in Cattle (1949)*), but a recent report (J. W. Thomas and S. Moss, *J. Dairy Sci.* **34,** *929 (1951)*) failed to reveal evidence of X-disease in young dairy bulls subjected to molybdenum intakes that produced severe toxicity symptoms (weight loss, anemia, greying of fur, mild diarrhea and lameness).

The implication that X-disease may involve a nutritional deficiency is apparent from the studies of the Cornell group. There are similarities between some of the lesions of X-disease and those seen in vitamin A deficiency (excess lacrimation, epithelial proliferative changes, abortion, or dead young at birth). Hansel, McEntee, and Olafson (*loc. cit.*) determined the plasma and liver carotene and vitamin A concentrations on 24 calves and one bull. The plasma vitamin A declined rapidly when the X-disease-producing agents were fed, and this decline preceded the other symptoms of the disease. The low plasma vitamin A levels persisted for a month beyond the period when the causative agents were fed. These workers suggest that the X-disease-producing agents may be regarded as exerting, in addition to other effects, an antivitamin-A effect and that this antivitamin-A effect is mediated at least in part by interfering with the conversion of carotene to vitamin A. This is based on their observation that in hyperkeratosis the vitamin A blood values declined more rapidly than occurs on vitamin A-depletion rations, whereas in the case of carotene the decline in blood levels was much slower than occurs when vitamin A-depletion rations are fed.

All mammalian species thus far studied (rabbits, sheep, rats, mice, guinea pigs, and cats) appear to be subject to the harmful effects of the agents that produce X-disease in cattle.

Reports of poisoning from chlorinated naphthalenes among industrial workers handling these compounds are numerous in the medical literature, according to L. Schwartz (*J. Am. Med. Assn.* **122,** *158 (1943)*). The human condition is referred to as cable rash, cable itch, or chloracne, and often also involves acute yellow atrophy of the liver. It was observed particularly among shipyard workers who stripped cables, or among electricians working with electrical wiring that was insulated with chlorinated naphthalenes.

It should be re-emphasized that causes for X-disease, other than contact with or the ingestion of chlorinated naphthalenes, may very likely be discovered.

To the nutritionist, the antivitamin-A effects of the chlorinated naphthalenes should be of great interest, since up to the present there is reference in the literature to only one antivitamin-A active substance, that obtained by the treatment of vitamin A with vanadium oxide (*Nutrition Reviews* **9,** *140 (1951)*).

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CHANGES IN BODY BUILD AND BODY COMPOSITION DURING AGING

While studies of growth and child development have been cultivated intensively, the slower and less dramatic but not unimportant alterations taking place in the adult man and woman have received little systematic attention. There have been, of course, the height-weight-age standards ("Association of Life Insurance Medical Directors and Actuarial Society of America, Medico-Actuarial Mortality Investigation," *Vol. 1, New York (1912)*). They have indicated, as did the tables compiled in other parts of the western world, an average trend toward an increase in body weight long after the cessation of the growth in stature.

Two large studies on age differences in body build were carried out by E. A. Hooton and his associates in the Department of Anthropology, Harvard University, and at the Quartermaster Corps. The two samples included some 45,000 U. S. Army separatees (40,000 of whom were whites), and 10,000 native-born Irish males, age 15 to 94 years.

In the Army series the external morphology of the men was described in the framework of a system of "somatotyping," derived from the work of W. H. Sheldon (Sheldon, S. S. Stevens, and W. B. Tucker, "The Varieties of Human Physique," *Harpers, New York (1940)*). The method involves the rating, on a scale from 1 to 7, of two "components" of body build—the fat (roughly an equivalent of Sheldon's endomorphy) and the bone-muscle (Sheldon's mesomorphy)—on the basis of a careful inspection of nude photographs, presenting the front, rear, and side views of each individual. The ratings were made first for four regions (upper and lower torso, upper and lower extremities) and averaged. The value of the third component labelled "attenuation" ("skinniness," elongation, approximately corresponding to Sheldon's ectomorphy) was assigned on the basis of a height-weight index.

The multiplicity of body "types" (*i.e.*, of different sequences of three-digit numbers) makes the presentation of results cumbersome. Furthermore, the soldier group cannot be regarded as a representative sample of the general population, and the selective process continues even after the induction. Also, army life itself is likely to modify the general age trends in body build. The normal process of development during adulthood seems to involve an initial loss of adolescent fat, gain in musculature during the early years of maturity, and fat increments beginning at a slightly later period and continuing well into middle age. Even in the Army material the fat "types" tended to increase in frequency with age. As one would expect, the third body-build "component," the "skinniness," was inversely related to age, the high values of skinniness being associated with young ages. However, some lean and muscular types are present at all ages.

Examination of the problems of body-build changes in terms of the somatotype frequencies (E. A. Hooton, *Am. Acad. Orthopedic Surgeons, Instructional Course Lectures* **8,** *147 (1951)*) is made very difficult by the low occurrence of the majority of the "types." Over 80 per cent of the types in the Army sample had frequencies of less than 1 per cent. The effect of random fluctuations is difficult to evaluate under such circumstances. At any rate, the multiplicity of the "body types" greatly complicates the analysis. Pooling of similar body types is only a partial remedy.

In order to overcome these technical difficulties, R. W. Newman (*Am. J. Phys. Anthropol.* **10,** *75 (1952)*) evaluated the data with reference to changes in "components." First, he calculated the frequency of occurrence of the ratings, varying from 1 to 7, for the three components. Within the age range from 18 to 35 years the per-

centage of high "fat" ratings (component 1) indicated a definite and marked increase throughout the range, with the rating "5" increasing from 8 to 28 per cent. In the second component ("muscularity") the trend was somewhat similar, but the differences were smaller (4 and 10 per cent in the rating "5"), reaching an early plateau. In the third component ("linearity," "skinniness") the high values decreased, "5" declining from 27 per cent at the age of 18 to about 12 per cent at the age of 35. This mode of evaluation is also not too satisfactory, because there are still too many variables (theoretically 7 curves for each component), even though not all ratings were actually considered, as the extreme values ("1" and "7") did not occur with sufficient frequency.

The simplest way to study the age trends is to compute the mean values of each component for the successive age groups. In these terms, the "fat" component increases from 4.1 to 4.8 (an increase of +0.7 from 18 to 35 years), component 2 rises from 3.6 to 4.1 (reaching a plateau at 27 years, with some indications of subsequent decrement), while the third component decreases (4.6 at 18, 3.8 at 35 years of age). This would then indicate definite changes in body build with age, due to an increase in the soft tissues, notably fat. The values are probably underestimates of changes taking place in the general population, as the men in the armed services engage, on the whole, in a greater amount of physical activity than they would in civilian life. This could account for some increment in muscularity. Also, the factor of selection would tend to make the increment in fatness with age more conservative, even though the induction fitness standards were not excessively high.

The Harvard Anthropological Survey of Ireland was conducted in 1935, but the results have been reported in detail only recently (E. A. Hooton, *loc. cit., p. 152*) Hooton and C. W. Dupertuis, "Age Changes and Selective Survival in Irish Males," *Studies in Physical Anthropology No. 2 (1951)*). A large number of measurements, but no body-type photographs, were taken. The sample was drawn from the rural population of all the Irish counties, with 14 age groupings (from 15 to 79 years of age at five-year intervals with the last group including ages 80 to 94).

The data indicate that many, and perhaps the majority, of morphologic features continue to change throughout the lifespan of an individual, an observation earlier documented by A. Hrdlička in his study on growth during adult life (*Proc. Am. Philos. Soc.* **76,** *847 (1936)*). Thus the breadth of the nose keeps on increasing during the whole adult existence. Many characteristics continue to rise during maturity and decrease during senescence. With reference to nutritional status, the Irish data on stature, and, in particular, on body weight are of interest in view of the scarcity of valid data for old age. In the 20 to 24 year group the stature is 172.6 cm., a maximum of 173.2 is reached in the third decade, with a decrease to 170.9 at 50 to 54 years of age and 165.8 at the age of 80 plus. The decline in stature is likely to be a true aging phenomenon, accounted for by less erect posture due to a lowered muscle tonus, increased curvature of the spine, and thinning of the intervertebral discs. Body weight, corrected for the weight of garments estimated about 7 pounds on the average, increased regularly from 145.8 pounds at 20 to 24 years to 156.9 pounds at 35 to 39 years, reached a maximum of 157.6 at 55 to 59 years and declined fairly rapidly in subsequent five-year groups, reaching 145.0 pounds in the oldest (80+) group. The decline in the means of men 60 years old and older is probably the resultant of two factors, true old-age shrinkage and selective mortality, favoring the survival of lighter men.

A tissue analysis of the age trends in body weight during maturity, necessary for a nutritional interpretation of the observed weight changes, was reported by J. Brozek

(*Fed. Proc.* **11,** *784 (1952)*). The body composition was characterized by means of skinfold measurements, used as an index of subcutaneous fat, and of specific gravity. The latter technic allows the separation of the gross body weight into body fat and the lean body mass. The subjects were selected in such a way that the mean relative weight of the samples (actual weight expressed as percentage of the standard weight for height, age and sex) closely approximated 100.

In samples with mean ages of 20.3, 25.2, 46.0, 50.0, and 54.6 years the average body fat accounted for 9.9, 14.4, 22.2, 24.0, and 25.2 per cent of the body weight. An equation for predicting the average fat percentage in adult men of different ages was presented.

Nutritionally, it is of interest that the increase in the fat fraction is faster than the increment in total body weight. Thus for a man 176 cm. tall the standard weight increases from 67.6 kg. at the age of 20 to 76.8 kg. at the age of 55 (a difference of +10.2 kg.), while the respective values of the estimated body fat are 7.0 kg. and 19.2 kg. (a difference of +12.2 kg.). At the same time, the lean body mass indicates a decrement from 60.6 kg. to 57.6 kg.

The decrease in the latter component is compatible with the known decrement in basal metabolism with age. In a sample of 33 young (22 to 29 years) and 33 middle-aged (48 to 57 years) men, matched for height and weight, the daily basal caloric expenditure corresponded to 1774 calories and 1516 calories, respectively. When the basal oxygen consumption was expressed in terms of the estimated "active tissue mass" (45.2 kg. and 37.9 kg.), the difference between the two age groups, amounting to 14.5 per cent on the basis of the calories per person, was markedly reduced. The value of "active tissues" was estimated as body weight minus the weight of fat, extracellular fluid, and bone minerals. The caloric expenditure per kilogram of active tissue mass was 41.7 and 40.0, yielding a difference of 4 per cent. While a difference still remains and may be considered as an indication of a real slowing down in the metabolic rate of the "active" tissues, the change during maturity is small.

The increase in body fat was interpreted as long-continued positive caloric balance. In the etiology of the decreased mass of the active tissue, interpreted principally as a decrease in muscle mass, a decrease in physical activity with age was emphasized. The tendency toward a lessened appeal of physical exercise and decreased participation in active games and sports was clearly established in a study of the behavioral and attitudinal differences between young and middle-aged men (J. Brozek, *J. Gerontol.* **7,** *410 (1952)*).

The effect of physical exercise on body composition was investigated (*Fed. Proc., loc. cit.*) by making a comparison of a group of normal middle-aged business and professional men who differed in the amount of habitual physical activity. The "active" group continued during adult age to participate in recreational activities involving a fair amount of physical exertion while the "inactive" group had always been relatively sedentary. The groups were matched for age (52.9 and 52.2 years) and height (176.1 and 176.2 cm.). The active individuals were heavier (81.5 versus 78.3 kg.) and would be labelled as "overweight." However, the excess weight was due to the lean body mass (61.7 versus 56.9 kg.), not to fat (19.8 versus 21.4 kg.). The difference in the lean body mass was statistically highly significant. These data were considered as confirming the thesis that the decrement of lean body mass, seen in the process of aging, represents essentially "disuse atrophy," with the loss in muscle weight being compensated for by the increase in the deposited fat.

Nutritionally, the principal conclusion drawn from the data was that the average caloric intake during maturity tends to be

larger than the actual caloric needs. While the Recommended Dietary Allowances (Revised 1948, *National Research Council Reprint and Circular Series No. 129, Washington, D. C.*) take into account sex and the activity level, no adjustment is provided for age. The "Calorie Requirements" (*FAO Nutritional Studies No. 5, Washington (1950)*) suggest a reduction of 7.5 per cent of the requirement for calories at the age of 25 years for each subsequent decade. On this basis the average requirement at 35, 45, 55 and 65 years would correspond to 92.5, 85.0, 77.5 and 70.0 per cent of the caloric requirement of a 25 year old individual (*Nutrition Reviews* **9,** *40 (1951)*). This is an approximate estimate and needs further empiric verification.

SODIUM RESTRICTION AND RETROLENTAL FIBROPLASIA

Retrolental fibroplasia, a disease causing blindness in small premature infants, remains one of the least understood complications of prematurity (*Nutrition Reviews* **8,** *353 (1950)*). The etiology of this disease remains unknown. Nutritional factors have been implicated in the past. The correlation between the incidence of this malady and large intakes of medicinal iron and certain multivitamin preparations (*Ibid.* **7,** *169 (1949)*) has not been a universal finding. The therapeutic effect of large doses of vitamin E (*Ibid.* **8,** *116 (1950)*) has also failed of confirmation. The failure of ACTH to prevent the development of blindness in affected infants (J. T. Lanman, L. P. Guy, and J. Dancis, *Pediatrics* **9,** *27 (1952)*) is another example of an ineffective therapeusis. During the decade since this disease was described by T. L. Terry (*Am. J. Ophth.* **25,** *203 (1942)*) its pathogenesis and clinical course have become much more clearly understood. The incidence of permanent ocular changes has been found to be less than one-third that of the early acute changes which have a marked tendency to regress spontaneously. The geographic and temporal variations in incidence, however, remain obscure.

A nutritional basis for this variation is postulated by W. R. Hepner and A. C. Krause (*Pediatrics* **10,** *433 (1952)*). Since retrolental fibroplasia is apparently rare in European countries and in certain hospitals where small premature infants are fed human milk exclusively, a small-scale study upon the effects of different milk feedings was carried out. Twenty-four infants weighing between 850 and 1540 g. at birth were fed either human or cow's milk formulas until they reached a weight of 1800 g., when all were fed the latter formula. Three cases of retrolental fibroplasia occurred in each group, but only after the transition had been made in those infants fed breast milk. All 12 infants retained fluid when transferred from human to cow's milk formulas. Comparisons between the electrolyte composition of the two feedings revealed that the cow's milk formula contained 10 per cent added carbohydrate which in turn contained 2 per cent sodium chloride. Thus the cow's milk formula had a sodium concentration of 56.4 mEq per liter, which was many times that of human milk (6.4 mEq per liter). Review of the feeding of extreme premature infants at the Chicago Lying-In Hospital revealed that the 45 per cent incidence of retrolental fibroplasia occurring in 1946–1949 was associated with this formula while previous and subsequent experiences with human milk or relatively dilute cow's milk formulas led to an incidence of only 7 per cent.

Evidence that infants who developed retrolental fibroplasia received more and larger transfusions of whole blood was also presented.

It is well known that the premature infant has a diminished ability to excrete

electrolytes such as sodium, particularly when the diet contains them in high concentration. Consequently, varying degrees of fluid retention and edema are common under these conditions. Blood transfusions for the correction of the anemia of prematurity are liberally given. Hepner and Krause point out that premature infants have poor pressor-receptor responses and might be expected to respond to large transfusions with hypertension and to compensate by the passage of fluid into the already overexpanded extracellular compartment. The frequency of vascular engorgement and tortuosity and retinal edema in the early stages of retrolental fibroplasia suggests to them an abnormality of physiology also expressed by the rounded body contours and cutaneous changes of premature infants receiving diets high in electrolyte content.

This hypothesis was also tested in kittens and has not yet been reported in detail. In this animal retinal changes similar to those seen in premature infants could be produced and maintained only in the presence of edema and large blood transfusions. Hepner and Krause are careful to point out that much of their hypothesis lacks support by adequate laboratory and animal studies, but that it is susceptible of clinical testing. It should also be pointed out, as summarized in the recent review by L. Zacharias (*Am. J. Ophth.* **35,** *1426 (1952)*), that retrolental fibroplasia has developed in infants who received only breast milk; other negative evidence is also available from the same survey. Nevertheless, it will be interesting to see whether or not further experience will bear out the importance of these nutritional and physiologic considerations in the etiology and treatment of retrolental fibroplasia.

PYRUVATE METABOLISM IN DIABETES AND LIVER CIRRHOSIS

Abnormalities of glucose metabolism, although implicit in diabetes mellitus, are not so well defined in cirrhosis of the liver. In this disease impaired tolerance to intravenously administered glucose has been reported by some investigators, but not observed by others. Even patients with hepatic coma do not necessarily have abnormalities of blood glucose levels or of tolerance to its administration. Normal tolerance to administered glucose, however, does not imply normality of carbohydrate metabolism. In a search for abnormalities of glucose breakdown measurement of blood pyruvate concentrations after glucose infusion were made by D. S. Amatuzio, N. Shrifter, F. L. Stutzman, and S. Nesbitt (*J. Clin. Invest.* **31,** *751 (1952)*).

Twenty-eight normal subjects served as controls in the study. Twenty-three patients with controlled diabetes mellitus and 15 with cirrhosis of the liver were compared with the controls with respect to blood pyruvate concentrations at intervals up to one hundred and eighty minutes after the intravenous administration of 0.5 g. of glucose per kilogram of body weight. Three of the patients with cirrhosis were in hepatic coma when the test was done.

In the normal subjects and the patients with "compensated" cirrhosis there was a small but statistically significant rise in blood pyruvate thirty and sixty minutes after glucose administration with a return to or slightly below normal at one hundred and twenty and one hundred and eighty minutes. In the cirrhotic patients the rise was from 1.08 to 1.49 mg. per cent.

The diabetic subjects showed quite different blood pyruvate curves. Instead of the rise seen in the normal subjects and the patients with cirrhosis (not in coma) after glucose infusion, there was either no significant change (mildly diabetic patients) or a

distinct fall at thirty minutes (severely diabetic patients).

Twelve units of insulin given to 11 of the control group and to 5 of the diabetic patients requiring insulin invoked a rise in blood pyruvate of greater magnitude (from 1.04 to 1.74 mg. per cent) than that observed after glucose administration in the normal subjects.

The 3 patients with hepatic coma demonstrated the most abnormal response. Following glucose administration the already somewhat high blood pyruvate rose progressively to values between 3 and 4 mg. per cent at one hundred and eighty minutes. The blood glucose concentration had returned to normal by one hundred and twenty minutes.

The progressive rise in blood pyruvate levels in the patients with liver coma was interpreted by the authors as a failure of the liver to "assimilate this metabolite." This is in agreement with the earlier work of D. S. Amatuzio and S. Nesbitt (*J. Clin. Invest.* **29,** *1486 (1950)*), in which elevated blood and spinal fluid concentrations of pyruvate were observed in hepatic coma.

The authors did not find a suitable explanation for the fall in pyruvate concentration of the severely diabetic patients after glucose administration, although it appeared related to the degree of insulin deficiency.

NUTRITION AND PREGNANCY

Demonstrable improvement in human health as the result of public health nutrition programs is not easily achieved and, because of variation in the national diet, satisfactory procedures in one area may not be applicable to another. Since much nutrition teaching rests upon theoretic probabilities, practical demonstration of their value is worthy of note. A recent study from Chile by A. Riquelme and R. Alvarado with the assistance of N. Segovia, S. Rosas, M. Malic, and R. Nieves (*Acta paediat. Española No. 113, 310 (1952)*) has confirmed the earlier studies of J. H. Ebbs, F. F. Tisdall, and W. A. Scott (*J. Nutrition* **22,** *515 (1941)*) and B. S. Burke, V. V. Harding, and H. C. Stuart (*see Nutrition Reviews* **4,** *175 (1946)*) of the positive relation between alimentation and outcome of pregnancy.

A group of women between 16 and 35 years of age in the third or fourth month of pregnancy were selected who were free of tuberculosis and cardiac or renal disease. They were divided into three groups, namely, a control group of 64, a group which received a dietary supplement (76 women), and a group which received nutrition education (106 women). The dietary supplement per day included dried milk equivalent to 500 cc., one egg, 50 g. of cheese, an orange or tomato, and a vitamin pill supplying 1.5 mg. thiamine, 2 mg. riboflavin, 15 mg. niacin, 5 mg. alpha-tocopherol and 800 I.U. calciferol. This supplement was continued for approximately six months. The outcome of each pregnancy was classified as good, regular, or poor, but the person making the classification, or whether each was made by the same person, is not specified. The classification was made by the obstetrician.

In the control group 64 per cent were classified as good, 31.2 per cent as regular, and 4.6 per cent as poor. In the supplemented group the figures were 75, 22.3, and 2.6 per cent, respectively, and in the group receiving nutrition education 85.8, 12.2, and 1.8 per cent, respectively. The increase in the "good" and decrease in the "regular" in both of the latter groups were statistically significant compared to the control group.

The weights of the infants were as follows: control, 2919 ± 43 g.; supplemented, 3334 ± 55 g.; and nutrition education; 3380 ± 53 g. The lengths of the infants in

the same groups were 48.2 ± 0.20, 49.9 ± 0.24, and 49.8 ± 0.28 centimeters.

The length of time of lactation was studied in the three groups. In the control subjects 30 per cent completed three months and only 7 per cent were lactating at five months. In the supplemented group 81 per cent lactated three months and 65 per cent lactated more than five. Similar figures were obtained for the groups which received nutrition instruction. Considering the total number of infants and the number of illnesses during the six months following birth, the illnesses per child per month were calculated. In the control group a value of 0.73 per cent was obtained compared to 0.37 per cent for those in the experimental groups, either supplemented or receiving education. It would appear from the report that the controls in this latter comparison may not have been the same group studied in the other sections, but simply a group of mothers and children who were studied in the Sanitary Unit of San Miguel.

The presentation of this report leaves considerable information to be desired. Although clinical examinations and nutrition histories of the subjects were made prior to the study, none of the findings is recorded. The extent of malnutrition is thus not recorded. Similarly, the type of education given the subjects is not specified and one does not know whether this was class teaching, family contacts, supervision during the entire period, or only occasional instruction, etc. The economic status of the group is not given, and the question as to whether the group is representative of the Chilean population is not clear. This is a particularly important question in view of the favorable results obtained with nutrition teaching. One supposes that the group was of low economic status but, even so, apparently had sufficient resources to improve their diet by more careful management. Dietary histories taken during the study would also have been of interest.

INFLUENCE OF INTESTINAL FLORA AND LIVER DISEASE ON CHOLINE ABSORPTION

Since choline is widely used and has been strongly advocated in the treatment of liver disease in man, it is encouraging to note that studies are under way to learn something of its absorption and intermediary metabolism. It will be remembered that J. de la Huerga and H. Popper (*see Nutrition Reviews* **9,** *351 (1951)*) found that most of the choline administered by mouth to either normal individuals or patients with biliary tract disease was transformed, presumably in the gastrointestinal tract, to trimethylamine, which then appeared quantitatively in the urine. Elucidation of these findings is reported by the same authors (*J. Clin. Invest.* **31,** *598 (1952)*), who found that aureomycin will decrease the degradation of choline to trimethylamine. The oral administration of choline in liver disease depressed the noncholine-utilizing flora with a concomitant increase in trimethylamine excretion. Finally, they found that patients with chronic liver disease appear to excrete far less trimethylamine than do either normal individuals, or patients with severe nonhepatic diseases, obstructive jaundice, or hepatitis, although there is some overlap in the last two groups with the reduced excretion found in cirrhosis.

The transformation of choline in the gastrointestinal tract was studied at different levels from the stomach through the small intestine by means of aspiration through a Miller-Abbott tube. In 3 patients with liver disease choline was transformed to trimethylamine at all levels except in the

duodenum. There seemed to be a rough parallelism between this transforming ability and the presence of bacteria.

It is interesting to note that prolonged daily administration of choline to 9 patients with cirrhosis brought the excretion of trimethylamine after a test dose of choline to normal. After discontinuance of choline treatment, the response of trimethylamine to a test dose of choline had again fallen to previous levels.

Finally, after the administration of lecithin there was a delay in trimethylamine excretion as compared to that after choline administration. Moreover, the degree of trimethylamine excretion after forty-eight hours was not so great after lecithin as after choline administration. In patients with cirrhosis of the liver, although there was a distinct reduction in trimethylamine excretion after choline administration as compared to normal individuals, the excretion of this substance after lecithin administration was perfectly normal.

The facts that (a) choline does not appear in the feces even after very large doses are administered by mouth, (b) that orally administered trimethylamine is excreted quantitatively in the urine, (c) that trimethylamine is not degraded by bacteria from the gastrointestinal tract *in vitro*, and (d) that there is a decrease in urinary trimethylamine excretion in patients with hepatic cirrhosis without the appearance of trimethylamine in the feces, all lead the authors to believe that there is an increased absorption of choline in chronic liver disease.

DIET AND THE METABOLIC DEFECT IN GOUT

The importance of dietary restrictions in the care of the patient with gout has historically been based upon more or less empiric grounds. Foods of high purine content have been eliminated in most therapeutic regimens on the basis that such foods yield uric acid precursors which are inadequately disposed of by the gouty patient. Recent investigations, however, have yielded information about uric acid formation and metabolism which, although not controverting the previous concepts of dietary therapy, tends to minimize the role of dietary purine in the production of overt gout. This newer knowledge of the metabolic defect in gout has recently been reviewed by DeW. Stetten (*Bull. N. Y. Acad. Med.* **28,** *664 (1952)*).

The use of N^{15}-labelled uric acid has made possible determinations of the size of the "miscible pool" of uric acid in normal subjects and gouty individuals and has also yielded approximations of the rate of generation of uric acid, or "turnover." In normal subjects fed purine-free diets adequate in protein, intravenous administration of N^{15}-labelled uric acid has revealed a miscible pool of uric acid of from 866 to 1341 mg. (average 1131). Approximately one sixth of this total uric acid pool appears to be in the plasma while the remainder is contained in the extravascular body water. However, appropriate plasma volume determinations and calculations have revealed that the uric acid concentration in some parts of the body water is lower than that normally found in the plasma.

When the extent of excretion of labelled uric acid in the urine is determined it is found that the turnover of uric acid takes place at a rate sufficient to replace 50 to 75 per cent of the uric acid pool daily with new uric acid. The uric acid thus replaced, however, does not all appear as such in the urine, but instead the finding of some N^{15} in urea and ammonia in the urine indicates that there are routes of uric acid loss or destruction other than its urinary excretion.

The site of this catabolism of uric acid is not exactly defined, but the gastrointestinal tract is probably a major participant since orally administered uric acid is more extensively broken down than is intravenous uric acid (W. Geren, A. Bendich, O. Bodansky, and G. B. Brown, *J. Biol. Chem.* **183,** *21 (1950)*).

In contrast to these findings in normal individuals are the results obtained in gouty patients in whom the uric acid pool is increased even in the absence of marked elevation of serum uric acid. Examples of the results obtained are a pool of 4742 mg. in a patient with mild gout, and a pool of 18,450 mg. in a patient with severe gout. In the latter subject it was possible to demonstrate an increase in the uric acid pool to 31,000 mg. after several months without treatment and a subsequent fall to 2084 mg. after four months of intensive therapy. These changes occurred without significant alteration in the serum uric acid concentration. The size of the miscible pool thus demonstrated in these patients indicates that not all of the uric acid in the pool is in a soluble state, but instead suggests that part of the urate in the solid gouty tophi is included in the pool. This has been confirmed by the finding of N^{15} in analyses of the outer layers of excised tophi but little or none in the central portions. Such results also suggest that tophi may be at least in part reversible, or redissolvable under proper circumstances.

The increased uric acid excretion produced by ACTH administration also has been investigated with the use of N^{15}-labelled uric acid and found to be renal in origin. The use of this hormone is accompanied by a decrease in serum uric acid without significant increase in the dilution of N^{15}-labelled uric acid in the serum which should result if ACTH were stimulating new uric acid production. Despite its usual protein catabolic properties ACTH is in this instance apparently without effect in stimulating endogenous uric acid production.

The most significant studies in regard to the specific metabolic defect in gout have been those of uric acid precursors. Glycine, which is known to enter the purine nucleus, has been labelled with N^{15} and given orally mixed with the diet. The appearance of N^{15} in the urine uric acid has then been followed as a measure of the glycine-purine-uric acid cycle. In the normal human subject the urine excretion of N^{15} in uric acid becomes maximum in three or four days and declines slowly with about 0.15 per cent of the N^{15} being excreted in uric acid in ten days. In gouty patients the N^{15} in urine uric acid rises to a maximum in two days to a level which is about triple that of normal subjects. The concentration then falls sharply with as much as 0.5 per cent of the N^{15} appearing in ten days. A greater amount of the labelled glycine thus appears to be converted to uric acid in the gouty person than in the normal human subject. In one patient so studied the use of a high protein diet with the labelled glycine yielded higher concentrations of N^{15} in the urine uric acid at all points than when a low protein diet was fed.

These results indicate that in the gouty individual a larger than normal fraction of dietary glycine is utilized for synthesis of uric acid. On the basis of the few studies available this metabolic defect is probably exaggerated by a high protein diet. With this knowledge dietary intake can be seen to be highly contributory to the gout syndrome, but the inherent metabolic defect is not correctable by dietary measures alone. The results obtained by A. B. Gutman and T. F. Yü (*Am. J. Med.* **13,** *744 (1952)*), in managing gouty patients with uricosuric agents plus diet restrictions, appear to demonstrate encouraging practical application of the newer knowledge reviewed by Stetten.

POTASSIUM, SODIUM AND CALCIUM IN GASTRIC SECRETIONS

The role of the gastric secretions in the digestion of ingested foods is of prime importance in the utilization of nutrients. Significant to this process is the secretion of juices which are higher in hydrogen-ion concentration than other biologic fluids and which possess great proteolytic activity. Investigations which were concerned with the mechanisms responsible for the elaboration by the gastric mucosa of secretions of high acidity have been the subject of earlier reviews (*Nutrition Reviews* **7,** *228 (1949)*; **8,** *199, 309 (1950)*).

Relatively little information is available concerning the concentration of cations other than hydrogen in gastric fluid and the changes in these with secretory activity of the stomach. Interest, therefore, attaches to the recently published observations of R. E. Bernstein (*J. Lab. Clin. Med.* **40,** *707 (1952)*), who has determined the concentrations of potassium, sodium and calcium in the gastric juice of normal human subjects under a variety of conditions of secretory activity. Quantitative determinations of cationic elements were done with the flame photometer utilizing an internal standard. The methods adopted were shown by the author to be more accurate and reproducible than standard chemical assays for sodium and potassium and of equal accuracy to the commonly used method for chemical determinations of calcium. Free and total acidity as well as the chloride content of gastric juice were also determined.

Specimens of gastric secretions were obtained from 50 healthy adult males. All subjects were studied first in the resting state after a night of fasting. Samples were taken at intervals of ten to fifteen minutes. Short intervals of collection were selected to limit the effect of back diffusion of ions and so that specimens contaminated with duodenal secretions as indicated by bile staining might be discarded. Efforts were made to prevent the swallowing of salivary secretions, since analysis of saliva had shown it to contain greater quantities of potassium and calcium than did gastric juice.

Specimens of gastric juice taken at these short intervals from the same subjects while resting were found to vary somewhat in content of free acid, sodium, and total chloride, while concentrations of potassium and calcium remained relatively constant. The mean values of 82 such specimens were 9.7 mEq per liter of potassium, 42 mEq per liter of sodium, and 3.3 mEq per liter of calcium.

Gastric secretory activity was stimulated in 14 subjects by administration of a solution of histamine subcutaneously, the dosage being 0.1 mg. per kilogram of body weight. Under this stimulus the volume, acidity and total chloride concentration of the secretion increased markedly to maximum values thirty to sixty minutes following the injection. In contrast, there was a decrease in the sodium, with values as low as 12 mEq per liter recorded in 3 patients. There was less effect on potassium levels, but the usual sequence was an initial increase with a fall to concentrations substantially below resting values in the period thirty to ninety minutes after the injection. Histamine stimulation of gastric secretion produced a profound inhibition of release of calcium into gastric secretions. In 8 of the subjects no calcium could be demonstrated in samples of gastric juice obtained at the height of the secretory response.

The response of the gastric mucosa to a test meal containing alcohol was also determined. The changes elicited were similar to those observed after histamine administration, with an increase in total acidity and decreased concentrations of sodium, potassium and calcium.

The effect of insulin on the secretion of

gastric juice was investigated. The quantity of the hormone injected is not stated, but was sufficient to produce a moderate decrease in blood sugar levels. With this stimulus there was increase in volume, acidity and chloride of the secretion. There was a consistent decrease in potassium, with most marked reduction sixty to one hundred and twenty minutes after the injection of insulin. Lowest values were recorded shortly after most marked reduction in blood glucose and plasma potassium had occurred. The changes in sodium concentration were similar to those observed after histamine injection, while calcium levels were relatively unchanged.

The injection of 0.6 to 1.0 ml. of a solution containing epinephrine hydrochloride in a dilution of one in a thousand effected a decrease in the volume of gastric secretion, as well as in total acidity and chloride. Calcium concentrations increased in all subjects, but levels of sodium and potassium were not changed significantly from those of specimens of gastric juice obtained from the same subjects in resting phases.

The interpretations which might apply to observations of this type are dependent on securing specimens free of salivary or duodenal secretions. The authors had taken precautions to avoid such contamination and had discarded specimens which were shown to contain other secretions. How well this objective was achieved is indicated by the relatively high correlation coefficient of about +0.9 between the values determined for free acidity and total chloride.

It is of interest that the concentration of potassium in gastric juice exceeded that in plasma under all conditions of secretory activity. However, the changes in concentration in gastric juice of this cation were not reflected in plasma potassium values. Similarly, there was no variation in plasma sodium content during active secretion. In contrast there was a consistently occurring decrease in plasma chloride during most active gastric secretion. The serum content of calcium was usually greater than that of gastric juice, but did not change during periods of major alteration of calcium concentrations in the secretion of the stomach.

The author interprets his observations in the light of current knowledge concerning the type of secretion elaborated by the various glandular elements in the gastric mucosa. Thus, the parietal cells are known to produce large volumes of an isotonic fluid containing hydrochloric acid and a smaller quantity of neutral chloride. The peptic and mucous glands produce a secretion of lesser volume containing mucin, pepsin and neutral chloride. Histamine stimulates the activity of the gastric mucosa with its chief response from the parietal cells. In contrast, the effect of insulin is to induce hypoglycemia with resultant stimulation of the vagus nerve and activity of all the glandular elements of the gastric mucosa.

The results of the experiments described by Bernstein (*loc. cit.*) indicate other differences in the response of the gastric mucosa to histamine and insulin. Histamine causes virtual disappearance of calcium from gastric secretions while calcium values are increased after insulin. This difference is explained on the basis that calcium is derived from cells other than parietal cells and presumably from mucous glands, since the viscous mucin of the gastric secretions contains greater quantities of calcium than does the entire gastric secretion.

Other investigations have indicated that a chemically equivalent amount of unidentified base must be given off to the vascular side of the secreting cell as acid is released into the lumen of the stomach. It had been presumed that sodium was the chief ion engaged in this interchange, but it is apparent from the results of the experiments described here that potassium must be involved as well.

These studies add new facets to the understanding of the composition of the digestive

juices of the stomach and contribute additional information concerning the mechanisms of gastric secretory activity. It is to be hoped that other observations will be made so that the full significance of the presence of these cations in stomach secretions may be appreciated and more adequately interpreted.

THE WORSENING WORLD FOOD SITUATION

During the period immediately preceding and following the end of World War II, considerable attention was given in the American press to the world's food situation. Pictorial illustrations of famine conditions dramatized for the public the state of nutrition prevalent among many large population groups. Lectures, movies and radio appeals reminded one at every turn that hunger was rampant again on almost every continent.

Little by little, the focus of interest changed from the social to the political. Average food consumption figures in Western Europe were known to have increased; by 1949 they appeared to insure at least maintenance of prewar values and probable continued progress. A number of international organizations, the Food and Agriculture Organization, the International Children's Emergency Fund, the World Health Organization, were known to be actively at work; only actual consideration of the insignificant amount of money allotted for technical assistance led to doubt that much could be accomplished toward rapid raising of the levels of world nutrition.

The authoritative statistical data on food production and consumption for 1951–1952 published at the end of 1952 by the Food and Agriculture Organization of the United Nations ("The State of Food and Agriculture: Review and Outlook, 1952," *FAO, Rome, Italy (1952)*) should go a long way toward dispelling this illusion of improvement on a world-wide scale. Briefly stated, the main conclusions which can be derived from comparison between the 1949–1950 and the 1951–1952 figures are as follows: The yearly expansion of food production of 1 per cent is, on a world-wide basis, at best barely keeping up with the increase in population. In addition, changes in consumption have taken place which have tended to make more acute the differences between "haves" and "have-nots." In the interval 1950–1952, the United States, with the highest caloric intake of any large population group and with overweight the number one nutritional problem, further increased the daily average caloric intake from 3170 to 3210 calories. Protein consumption increased from 91 to 93 g., animal protein consumption from 61 to 62 g. per day. India during the same years reduced its daily caloric consumption from 1620 to less than 1600, almost 400 calories below the prewar average. The individual's total protein consumption, at 43 g. per day, is 13 g. below the prewar level; animal protein, at 6 g. per day, is 2 g. below the prewar average.

To appreciate the full significance of these facts, consideration of long-range trends is essential. In a review published three years ago, C. Chatfield, M. L. Scott, and J. Mayer (*Milbank Memorial Fund Quart.* **28,** *103 (1950)*; *Nutrition Reviews* **9,** *40 (1951)*) analyzed the changes in world food consumption over the 1938–1948 decade. During this period, the world population increased from 2,174 to 2,354 millions, an increase of 8.3 per cent. More than half of the world population is found in Asia (1,247 millions in 1948, not including Siberia). The Asian populations during that decade increased by 8.2 per cent, similar to the general average. Greatest rates of increase were found in the Americas (North America, 13.5, Central America, 22.0 and South

America, 20.7 per cent). Europe (3.5 per cent) and USSR (2.6 per cent) showed the slower rates.

During the 1938–1948 decade, the over-all world-wide production of calories available for human consumption increased by 0.7 per cent, that of total proteins by 1.4 per cent; vegetable protein, 1.2 per cent; animal protein, 1.9 per cent and "milk equivalent" by 6.2 per cent. None of these increases was sufficient to match the rate of growth of the human population. Consequently caloric consumption per head during that decade dropped by 7.1 per cent; total protein by 6.4; vegetable protein by 6.7; animal protein by 5.6 and "milk equivalent" by 1.5. Furthermore, inequalities in consumption were made more acute. For example, the United States consumption figures for milk equivalent increased from less than 600 to nearly 800 ml. per day while for more than 30 per cent of the world's population consumption dropped to less than 10 ml. per day.

By and large the two- or three-year period following this 1948 review showed definite improvement in many regions of the world which were recovering from World War II destruction. If it had been possible to maintain the same rate of increase during the following 1951–1952 period, there would be less ground for pessimism. The comparison between 1949–1950 and 1951–1952 values given by the FAO report showed, however, that nutritional improvement in most poor regions stopped after 1950. Besides the example of India which has been given above, the completely stationary state of caloric and nutrient intakes throughout South America is particularly noteworthy. In Denmark, Germany, Austria, Greece, Ireland and Italy caloric and nutrient intakes are below the prewar averages. Several countries, the United Kingdom in particular, show a downward trend. In the Far East, virtually every country is below the prewar average. After some improvement, Japan and Indonesia show a downward trend in the quality of the diet; India and Pakistan a decrease in both quality and quantity. The 1938–1948 trend is therefore again developing.

The FAO report analyzes the reasons for this situation. Many countries have tended to increase the agricultural production of nonfood products at the expense of food production. The price rise following the Korean outbreak has limited the buying capacity of populations in many areas and has tended to cause an evolution of the ratio of costs to price in a direction unfavorable for farmers. The ever-increasing barriers restricting international trade have grown at an "alarming rate," affecting the demand for many of the nonbasic foodstuffs and forcing a continuation of austerity in many parts of the world. Dollar shortages, interruption of movement of food and agricultural machinery, and restriction of private investments in agriculture and in expansion of underdeveloped areas have also resulted from barriers in international trade. Finally, unsettled conditions in some of the rice-exporting countries (Burma exports only one third of the prewar average; Indo-China exports hardly any rice at the moment) add to difficulties in the Far East.

If greater knowledge leads to increased responsibility, then nutritionists have a special duty to inform the public on the nutritional situation of the world. Present conditions justify neither complacency nor relaxation of efforts in the fields of international trade or technical assistance.

VITAMIN CONTENTS OF SWEET POTATOES RELATIVE TO STORAGE CONDITIONS

Since sweet potatoes are staple foods ordinarily available from storage, it is important to know the changes in nutrient values to which they are subject during various storage conditions. Sweet potatoes are third in dollar value among our vegetable crops, being exceeded only by white potatoes and tomatoes. The yellow varieties are good sources of provitamin A and all are good sources of ascorbic acid. Several comprehensive studies have recently been made to determine the effects of storage conditions on vitamin contents, sprouting and other quality factors.

Pre- and postharvest changes in carotene (provitamin A), total carotenoids and ascorbic acid (vitamin C) contents of sweet potatoes were reported by B. D. Ezell and J. N. Crowder (*Plant Physiol.* **27,** *355 (1952)*). Four varieties, differing widely in color, were grown at Beltsville, Maryland, and analyzed throughout the growing season and later. Size of root was not considered a major factor in determining the carotenoid content. This study extended over a longer period than the commercial harvest season, which extends from the date of earliest frost expectation to ten days after the average date of first frost.

Although the more deeply colored varieties (Porto Rico and Orange Little Stem) increased in carotenoid pigments more rapidly than the paler ones (Yellow Jersey and Nancy Hall), both carotene and total carotenoids increased in all varieties as the season advanced, reached a maximum, and then declined later as climatic conditions became less favorable for growth. Except for Yellow Jersey, the same was true of ascorbic acid contents. In this case, no increase occurred, but the late decrease did occur. Carotene in the two lighter varieties varied between 0.2 and 1.7 mg. per 100 g. and in the darker varieties from 3.5 to 5.8 mg. per 100 g.

The time of harvest profoundly affected the further synthesis of carotenoids during storage at 60°F. Chilling before harvest of the late-harvested Yellow Jersey roots permanently impaired the mechanism for synthesis of carotenoid pigments. Early and middle harvest roots continued to increase considerably in carotenoid content for several months after harvest. Nancy Hall roots did not show this increase, nor was it so striking in the other varieties. The maximum concentration of carotene occurred at about the time of the usual commercial harvest. Ascorbic acid decreased in all cases about 50 per cent to a relatively constant value of 15 to 25 mg. per 100 g., irrespective of time of harvest.

In sweet potato roots, differences in flesh color are due mostly to differences in carotene content. All varieties had approximately the same amounts of noncarotene carotenoids throughout the season and these values were low. It appears that the failure to synthesize carotenoid pigments, or a reduced rate of synthesis in the roots after harvest, is a very sensitive measure of physiologic injury of sweet potatoes.

These studies were extended by B. D. Ezell and M. S. Wilcox (*Plant Physiol.* **27,** *81 (1952)*) to include the influence of storage temperature. Temperatures of 50, 55, 60 and 70°F. were studied. Carotenoid changes are very sensitive to storage temperature differences. At 50°F. there was no appreciable increase in any variety and Orange Little Stem showed a marked decrease. At 55°F. changes were slight. This is the temperature usually recommended. At 60°F. there was an increase of distinct nutritional importance in all varieties but Nancy Hall, a variety which lost carotene (and total carotenoids) at all temperatures studied. At 70°F. carotene increased less rapidly than at 60°F. except in Yellow Jer-

sey, in which it increased still more rapidly than at 60°F. This variety contained the least carotene at the start of storage. Losses of ascorbic acid were approximately equal and uniform for all temperatures and varieties.

The variety is very important in determining carotene content during storage. A suitable genetic background is necessary to provide a capability for carotene synthesis when environmental conditions are favorable. Quite late in the storage period (after five months) great losses of carotene occur.

The postharvest increase in carotene in sweet potatoes appears analogous to the postharvest increase in lycopene that occurs in tomato fruits harvested before fully ripe. The noncarotene fraction probably includes pigments in the intermediate stages of both synthesis and degradation of carotene, together with whatever xanthophylls may be present.

The sweet potato root would seem to be a favorable subject in which to study the mechanisms of carotenoid synthesis. More should be learned of the identity of the carotenoids present.

RELATION OF VITAMIN B_{12} TO COBALT DEFICIENCY IN SHEEP

Prior to the discovery of vitamin B_{12} in 1948, it was generally believed that cobalt was an essential nutritional factor only for ruminants, since a deficiency of this mineral could be produced only in cattle and sheep. Attempts to produce cobalt deficiency in other species were unsuccessful (J. F. Thompson and G. H. Ellis, *J. Nutrition* **34,** *121* (*1947*)). Since vitamin B_{12} contains cobalt, and since this vitamin is undoubtedly required in minute amounts by many, if not all, higher animals, it is now generally recognized that cobalt should be considered essential for all higher animals. Owing to the vigorous bacterial synthesis in the rumen or alimentary tract of ruminants, the demonstration of a need for vitamin B_{12} in such animals has been difficult.

Evidence is now accumulating to suggest that the deficiency of cobalt in ruminants is a manifestation of vitamin B_{12} deficiency and that cobalt is incorporated into vitamin B_{12}-active substances, probably by the rumen microorganisms. P. H. Abelson and H. H. Darby (*Science* **110,** *566* (*1949*)) were able to demonstrate radioactive cobalt in the fecal vitamin B_{12} when radioactive cobalt was fed to sheep. W. H. Hale, A. L. Pope, P. H. Phillips, and G. Bohstedt (*J. Animal Sci.* **9,** *414* (*1950*)) noted that rumen ingesta from cobalt-deficient sheep contained less vitamin B_{12} than the ingesta from cobalt-fed sheep.

D. E. Becker and S. E. Smith (*J. Nutrition* **43,** *87* (*1951*)) presented evidence that an antipernicious anemia liver extract gave a positive curative effect in cobalt-deficient sheep. Later Smith, B. A. Koch, and K. L. Turk (*Ibid.* **44,** *455* (*1951*)) found that cobalt-deficient sheep responded to pure vitamin B_{12} injections if large enough doses were given (150 micrograms or more per animal). The failure of these and other workers to obtain favorable responses from vitamin B_{12} in earlier attempts was believed to have been due to the use of inadequate amounts of the vitamin.

L. S. Gall *et al.* (*Science* **109,** *468* (*1949*)) reported an altered bacterial flora in the rumen of cobalt-deficient sheep. Somewhat earlier S. N. Ray, W. C. Weir, A. L. Pope, and P. H. Phillips (*J. Nutrition* **34,** *595* (*1947*)) had reported lower blood levels of certain B-vitamins (pyridoxine, niacin, and riboflavin) in cobalt-deficient sheep, which was suggestive of a disturbed rumen function. It was further reported that a complete B-vitamin supplement, exclusive of vitamin B_{12}, reversed the symptoms of

cobalt-deficiency in sheep (W. H. Hale, Pope, Phillips, and G. Bohstedt, *J. Animal Sci.* **9,** *484 (1950)*), an observation that could not be confirmed by H. A. Keener, G. P. Percival, G. H. Ellis, and K. C. Beeson (*Ibid.* **9,** *404 (1950)*).

The status of cobalt-deficient sheep with respect to rumen synthesis, blood levels, and liver storage of vitamin B_{12}, and to the niacin and riboflavin content of rumen ingesta, has been investigated by W. G. Hoekstra, A. L. Pope, and P. H. Phillips (*J. Nutrition* **48,** *421 (1952)*). These workers found that early in cobalt deficiency the blood of sheep contained only about one-fifth the vitamin B_{12} activity (as determined with *Lactobacillus leichmannii*) as did the blood of cobalt-fed sheep. In deficient animals the blood contained an average of 0.47 millimicrograms of vitamin B_{12} per milliliter. This was somewhat lower than the values of 0.6 to 0.9 millimicrograms per miililiter found in cobalt-deficient sheep by J. R. Couch, O. Olcese, P. W. Witten, and R. W. Colby (*Am. J. Physiol.* **163,** *77 (1950)*). Hoekstra and associates found that the blood of cobalt-supplemented sheep contained an average of 2.3 millimicrograms of vitamin B_{12} per milliliter and their results suggest that vitamin B_{12} blood levels in ruminants are dependent upon the intake of cobalt.

Liver storage of vitamin B_{12} was very low in the cobalt-deficient sheep; about thirty times more "true" vitamin B_{12} activity was present in the livers of cobalt-fed sheep. The "true" vitamin B_{12} activity was calculated by deducting the activity for *L. leichmannii*, after alkali treatment, from the total activity, and was found to average 0.025 micrograms per gram of moist liver in the deficient animals compared with an average of 0.92 micrograms for liver from cobalt-fed sheep.

Hoekstra and associates recognized the reservations that must be considered in accepting vitamin B_{12} values obtained by the *leichmannii* assay method. Vitamin B_{12f} and pseudovitamin B_{12} are active for this organism; the former is reported to be inactive for the rat, while the latter is inactive for chicks, rats and man. Despite these limitations, the differences found were so great that it was apparent that the cobalt-deficient sheep were severely limited in vitamin B_{12} as compared with sheep fed cobalt.

Rumen ingesta likewise were found to be markedly different. From cobalt-deficient sheep, these averaged 0.09 micrograms of vitamin B_{12} activity per gram dry weight, contrasted with an average of 1.3 when cobalt was fed.

The levels of niacin and riboflavin per gram of dry rumen material were essentially the same in cobalt-deficient and cobalt-fed sheep. However, the former animals were on a much lower nutritional plane. They had greatly decreased rumen contents, which would mean considerably lowered quantities of these vitamins and could explain the lowered concentration of these vitamins in the blood as noted in earlier studies.

The response of cobalt-deficient sheep to intravenously administered vitamin B_{12} was also investigated by W. G. Hoekstra, A. L. Pope, and P. H. Phillips (*J. Nutrition* **48,** *431 (1952)*). Three sheep that were severely deficient in cobalt, as evidenced by depressed appetite, body weight loss, and hemoglobin values of 4 to 7 g. per cent, exhibited definite improvement upon the injection of 20 micrograms of vitamin B_{12} twice daily for twenty-one days. The sheep gained vigor rapidly during the first week and gained an average of 40 pounds in body weight during eighty days following the initiation of treatment. The hemoglobin responses were somewhat delayed, but in all cases returned to a normal value of 11 g. per cent or more within forty to sixty days after vitamin B_{12} injection was begun.

The response to the above treatment continued for about one hundred days. At this time, when symptoms of a second cobalt

deficiency were evident, 2 of the sheep were given a second series of vitamin B_{12} injections (40 micrograms per head daily for twenty-one days). Excellent responses were again noted with respect to body weight recovery and general vigor. Hemoglobin responses were erratic. However, this was not considered unusual since delayed and erratic responses are also often seen after the administration of cobalt to cobalt-deficient sheep.

The fact that vitamin B_{12} injections brought about such excellent responses in sheep that had been on a cobalt-deficient ration for over five hundred days led these workers to the view that this vitamin is the most important, if not the only limiting factor, in cobalt-deficient sheep. The view that cobalt in an organically bound form is the active curative factor in cobalt deficiency is, of course, supported by the well-established observation that inorganic cobalt injected into cobalt-deficient animals is of little or no benefit.

Whether or not cobalt-containing compounds, other than vitamin B_{12}, will assume importance in ruminant nutrition will no doubt be of interest. Natural materials are known to contain a variety of substances of the vitamin B_{12} group. J. E. Ford (*Brit. J. Nutrition* **6,** *324 (1952)*) has recently reported the presence, in calf gut contents and feces, of three substances that had varying activities based on microbiologic assays. Two of these were found to be red cobalt-containing pigments, but none of them was convertible to vitamin B_{12} by the action of cyanide.

SERUM ZINC IN HEALTH AND DISEASE

The presence of zinc has been demonstrated in all human organs and tissues examined (B. L. Vallee and M. D. Altschule, *Physiol. Rev.* **29,** *370 (1949)*). It has been shown to be essential for the growth of experimental animals (*Nutrition Reviews* **8,** *239 (1951)*), and is known to be an active component of the carbonic anhydrase molecule. C. G. Holmberg's data have been interpreted by others to imply that zinc is part of uricase (*Biochem. J.* **33,** *1901 (1939)*). The much debated relationship of zinc to insulin and pancreatic function is far from unequivocal (Vallee and Altschule, *loc. cit.*). It has been claimed to be part of the enzyme uricase, and to be necessary for the storage of insulin in the pancreas.

R. A. McCance and E. M. Widdowson have shown (*see Nutrition Reviews* **1,** *411 (1943)*), by a balance study on human subjects, that the body can eliminate zinc administered in excess of the normal intake. The sensitivity of the regulating mechanism to a given dose varies with the individual and possibly with the degree of saturation of his storage organs. A series of experiments involving the use of radioactive zinc, in particular those of Vallee and his collaborators (*loc. cit.*), have shown that Zn^{65} disappears for the most part from plasma during the first twenty-four hours. Some of the radioactive zinc, however, could be detected in the blood of the patients for several weeks after the injection. Similarly, F. G. Ebaugh and J. F. Ross (*Fed. Proc.* **11,** *413 (1952)*) have found that after intravenous injection of radioactive zinc to patients with neoplastic diseases, only 11 per cent of the administered dose remained in the plasma after thirty minutes.

The binding of zinc to serum proteins has also been studied. D. M. Surgenor, B. A. Koechlin, and L. E. Strong (*J. Clin. Invest.* **28,** *73 (1949)*) have shown that zinc may combine the beta 4 VII (iron-binding) globulin (*Nutrition Reviews* **10,** *210 (1952)*) *in vitro*, but the physiologic significance of this observation is very questionable.

While numerous claims have been advanced on the correlation of variations of

serum zinc with pathologic conditions, the only substantial studies on the subject, until recently, have been those of Vallee and his collaborators, bearing principally on disorders of the blood (B. L. Vallee and J. G. Gibson, *Blood* **4,** *455 (1949)*; Vallee, H. D. Lewis, M. D. Altschule, and Gibson, *Ibid.* **4,** *467 (1949)*; Gibson, Vallee, R. G. Fluharty, and J. E. Nelson, *Acta unio internat. contra cancrum* **6,** *1102 (1950)*). F. L. Hoch and Vallee have recently reported the isolation of zinc-containing proteins from human leukocytes (*J. Biol. Chem.* **195,** *531 (1952)*; *J. Clin. Invest.* **30,** *650 (1951)*).

H. Wolff (*Deutsch. Arch. f. klin. Med.* **197,** *263 (1950)*) reports normal serum zinc values varying from 137 to 284 micrograms per cent. His mean values were 197 micrograms per cent for 25 men and 194 micrograms per cent for 25 women. The data were not treated statistically. A recent comprehensive publication gives results of extensive studies on zinc in blood in health and disease (I. Vikbladh, *Scandinav. J. Clin. Lab. Invest.* **3,** *Suppl. No. 2 (1951)*). Several papers have also appeared extending the available information and adding new data. Vikbladh reports mean serum zinc levels of 124 micrograms per cent obtained on 100 individuals. The standard deviation was ±15.9 micrograms of zinc per 100 ml. These latter values are much lower than those reported by any other author. Both authors utilized the dye dithizone as the colorimetric agent for zinc determination, though their technic differed somewhat. The data of both authors confirmed earlier reports which showed no sex differences, though the absolute levels of their series differed. Vikbladh reports the mean value of the serum zinc level in 16 newly delivered women as slightly lower than that of her normal series, that of their infants slightly higher than the normal average. By contrast, the zinc content of the erythrocytes of the newborn infants was only 25 per cent of the normal adult value. This observation correlates with observations of the low level of red blood cell carbonic anhydrase previously noted in premature and newborn infants (S. S. Stevenson, *J. Clin. Invest.* **22,** *403 (1943)*).

A publication by R. Berfenstam (*Acta paediat.* **41,** *Suppl. No. 87 (1952)*) contributes little to the existent facts and confirms Vikbladh's observation that the infant's serum zinc level at birth is higher than that of the mother's while the situation with regard to erythrocytes is reversed. The infant obtains large quantities of zinc from maternal milk, a large percentage of which is excreted in the feces, however. Increased serum zinc levels in female rabbits led to temporarily increased serum zinc levels in the offspring. The erythrocyte zinc content could also be increased by the intravenous administration of the metal without concomitant increase in carbonic anhydrase activity. This discrepancy was to be expected in view of the findings of R. Tupper, R. W. E. Watts, and A. Wormal (*Biochem. J.* **50,** *429 (1952)*), who demonstrated that Zn^{65} does not exchange *in vitro* with that contained in carbonic anhydrase.

Vikbladh also studied serum zinc levels in a number of acute infections, in particular malaria, pneumonias, acute rheumatic fever, erysipelas, thrombosis, pyelonephritis, acute dermatitis, serum sickness and bronchitis. A definite negative correlation was established between serum zinc content and body temperature. The zinc levels returned to normal as soon as the patients recovered.

Vikbladh also studied a number of anemic conditions. In 15 cases of posthemorrhagic anemia in benign diseases, a slight decrease in the serum zinc levels was observed. However, the difference from normal values was not significant. Eight cases of microcytic anemia with low serum iron values which responded rapidly to iron therapy (sideropenic anemia) had normal serum zinc levels. By contrast, 14 cases of pernicious anemia were found to have low zinc levels. All tests performed before liver therapy showed low

serum zinc values (between 70 and 110 with an average of 86 micrograms per cent) without exception. Twenty-four hours following the first injection of liver, the zinc content began to rise, returning to normal values during the first week. In pernicious anemia in remission (13 cases), low zinc values were not observed; no explanation is offered for this remarkable difference. "Aplastic" and "achrestic" anemias were accompanied by low, normal or high serum zinc levels without discernible trends or correlations between the type of anemia or the magnitude of the zinc level. This author reports four cases of hemolytic anemia, one of which had low while the other three had normal serum zinc levels. H. Wolff and R. Amann (*Klin. Wchnschr.* **29,** *316 (1951)*), by contrast, contend that hemolytic anemias are accompanied by "characteristically increased zinc levels."

Vikbladh's claim of low serum zinc levels in leukemia is unconvincing on the basis of her own data. Similarly unconvincing are the statistical interpretations placed on results obtained on patients with chronic rheumatic polyarthritis, liver disease, hypothyroidism and nephritis. She concludes that no marked differences from normal values could be observed in subjects with acute rheumatic fever, ulcerative colitis, diabetes, acute nephritis, peptic or duodenal ulcers. Results on a few individuals with widely differing diagnoses are so sketchy as not to permit any conclusions. In 24 cases of malignancy lowered serum zinc levels were observed.

Both Vikbladh and Berfenstam investigated the linkage between zinc and plasma proteins of normal blood. Vikbladh interprets her data to demonstrate a "firmly" and a "loosely" bound serum zinc fraction. The firmly bound zinc amounted to about 34 per cent while the loosely bound zinc amounted to about 66 per cent of the total serum zinc, the latter being responsible for the changes observed in pathologic states. She interprets her metabolic studies to indicate that this loosely bound fraction is identical with the beta 4 VII (iron-binding) globulin (B. A. Koechlin, *J. Am. Chem. Soc.* **74,** *2649 (1952)*; C.-B. Laurell, *Acta physiol. Scandinav.* **14,** *Suppl. 46 (1947)*). This contention is poorly substantiated by her data. Berfenstam's results add nothing but further confusion to this picture.

The increased attention to the physiology of zinc and its interaction with proteins is a consequence of vastly improved methodology permitting quantitative studies on body constituents existent in low concentrations. It may safely be expected that much of importance will soon be learned from the study of this and other trace metals.

CITRIC ACID METABOLISM IN BONE

The importance of citric acid in the metabolism of bone was indicated when it was shown that more than 90 per cent of the citric acid in the animal body is in the bony structure (F. Dickens, *Biochem. J.* **35,** *1011 (1941)*). It had been ascertained previously that combinations of calcium, phosphate and citrate in solution under suitable conditions form a precipitate not unlike that in which calcium is found in bones (A. C. Kuyper, *J. Biol. Chem.* **123,** *405 (1938)*). The solubility of this complex is increased by addition of magnesium citrate.

A variety of experimental findings demonstrate dynamic relationships between the metabolism of calcium and citrate. G. Gomori and E. Gulyas (*Proc. Soc. Exp. Biol. Med.* **56,** *226 (1944)*) found an outpouring of calcium in the urine when solutions of citrate were injected into dogs. In addition, there was evidence of decalcification of bone in these animals. The increase

in serum calcium induced by parathormone is associated with increased quantities of citrate in serum. Similarly, it has been shown that the serum citrate of infants with rickets is reduced, and increases markedly when large doses of vitamin D are administered (*Nutrition Reviews* **10,** *233 (1952)*).

Experimental evidence supports the view that processes of calcification are conditioned by the quantity of citrate in blood or at sites of bone formation. T. F. Dixon and H. R. Perkins (*Biochem. J.* **52,** *260 (1952)*) have attempted to evaluate the roles of certain enzymes important in the citric acid cycle on the availability of citrate in bone and thereby on mechanisms of calcification.

In a first experiment the effect of citrate on *in vitro* calcification of bone slices from rachitic rats was determined. It was found that addition of 0.0005 molar sodium citrate to the medium caused marked inhibition of calcification. This inhibition apparently resulted from binding of a portion of the ionized calcium. It was demonstrated, therefore, that an excess of citrate limited the availability of calcium for formation of new bone.

Dixon and Perkins proceeded to an investigation of the relative activities in bone and other tissues of certain enzymes which participate in the metabolism of citric acid. The systems studied included citrogenase, aconitase, and isocitric dehydrogenase (*Nutrition Reviews* **9,** *44 (1951)*). The term citrogenase, as used here, relates to the enzymatic reactions responsible for the formation of citrate from oxaloacetate and acetate. This conversion involves reactions forming acetyl phosphate from acetate, transferal of the acetyl group to coenzyme A, and condensation of the acetyl group with oxaloacetate to form citric acid (J. R. Stern and S. Ochoa, *J. Biol. Chem.* **191,** *161 (1951)*). The citric acid produced in the over-all reaction was determined after incubation of minced tissue or chopped bone from rabbits in a suitable medium containing the required substrates and adenosinetriphosphate. It was found that the tissues themselves contained adequate quantities of coenzyme A.

Aconitase activity was assessed by determination of the citric acid produced by tissue extracts from a substrate of cis-aconitate. Isocitric dehydrogenase in tissue minces was determined by reduction of dyes acting as hydrogen acceptors in a medium containing citric acid. It was found that the tissues contained sufficient aconitase to afford adequate conversion of citrate to isocitrate.

Comparison of the activities of these enzymes in various tissues showed that citrogenase activity of rabbit kidney was about twice as great as that of liver. The activity in bone was very much less than in kidney or liver. In bone, citrogenase was present with greatest activity in the metaphysis, where it was approximately one-fourth as active as in kidney. The enzyme was of lowest concentration in the cortex of bone, with but one-tenth the activity of the metaphysis or epiphysis.

Relative activities of aconitase were similar, with largest quantities of the enzyme in the kidney. In bone, the metaphysis and epiphyseal cartilage possessed greatest activity, about one-tenth that of kidney, while the quantity of this enzyme in the cortex was extremely low. However, aconitase activity of bone exceeded that of either of the other two enzymes determined.

The oxidative enzyme, isocitric dehydrogenase, possessed activity equal to that of citrogenase in tissues from kidney and liver, but was only one-tenth as active as citrogenase in the metaphysis and epiphysis of bone. No isocitric dehydrogenase could be demonstrated in the bony cortex.

Relative activities of these enzymes in bone favor synthesis of citric acid as compared to degradation and removal. Greatest

quantities of the enzymes responsible for formation of citric acid, citrogenase and aconitase, were found in areas where calcification was proceeding actively. These conditions would seem to be those which would permit the accumulation of citrate in local areas of new bone formation.

Interpretation of these observations in light of other knowledge of mechanisms of calcification is not easily achieved. It might be reasonably concluded that the increased concentrations of citrate made available at sites of new bone formation facilitates the deposition of calcium. On the other hand, citrate may, by binding calcium, limit its availability for deposition in bone, or, if present in excess, may act to mobilize calcium and by this means cause dissolution of bone.

The interrelationships between hormones, enzymes and nutritional factors in mechanisms of calcification are indeed complex. The experiments described do afford a new approach to the dynamic equilibriums involved, however. It is hoped that they will be elaborated further in the effort to provide new facets to our knowledge of bone formation.

THE EFFECT OF THYROXINE AND THIOURACIL ON CONSTITUENTS OF MILK

Many investigators have studied the effect of thyroxine and thiouracil on milk. Recently (*Nutrition Reviews* **11,** *19 (1953)*) R. Chanda and E. C. Owen discussed the effect of thyroxine and thiouracil on the vitamin A and carotene content of milk. Further studies by this group (Chanda, M. L. McNaught, and Owen, *Biochem. J.* **51,** *543 (1952)*) in Ayr, Scotland, dealt with the concentration of water-soluble vitamins and other constituents of milk as influenced by thyroxine and thiouracil.

These experiments were conducted in a manner similar to the other studies. In each experimental group control, thyroxine-treated, and thiouracil-treated cows were tested for three periods of three weeks each, receiving the thyroxine and thiouracil respectively only for the middle period. The following items were studied: milk yield, fat, nonfat solids, protein, lactose, chloride, freezing point, calcium, magnesium, sodium, potassium, creatine, creatinine, riboflavin, thiamine, phosphatase, cocarboxylase and ascorbic acid.

Thiouracil, 20 mg. per cow per day subcutaneously, decreased the milk yield of cows by 10.7 per cent, while DL-thyroxine, 10 mg. per cow per day subcutaneously, increased it by 13.2 per cent.

Thyroxine caused an increase in the fat of milk in some cows, and thiouracil caused a decrease. The effect of thyroxine on the nonfat solids was variable, as was the effect of thiouracil. Neither thyroxine nor thiouracil had any effect on the protein content of the fat-free milk.

There was a small increase in the lactose content of milk in the thyroxine-treated cows corresponding to a simultaneous decrease in the chloride content. Thiouracil caused the reverse effects. Since lactose and chloride account for most of the osmotic pressure of milk there tends to be a constant relationship between these two constituents. From analysis of numerous milk samples it was found that this relationship can be expressed by the following formula: lactose content $= 6.26 - 13.5 \times$ chloride content. The freezing point of milk was not changed by either drug.

The calcium, sodium, potassium, magnesium, protein, creatine and riboflavin contents of the milk were not markedly altered by either thyroxine or thiouracil. Daily values for hemoglobin, red blood cells and white blood cells could not be related to either compound. Neither thyroxine nor thiouracil caused any change in the content of creatine or creatinine. Cre-

atine in milk decreased with advancing lactation. Creatine phosphate was not found in the milk.

Thyroxine decreased the ascorbic acid content of milk by 25 per cent, while thiouracil caused an increase of 24 per cent. When the dosage of thiouracil was decreased by one-half, the increase in ascorbic acid was only 10 per cent.

Thyroxine did not affect the total thiamine content of the milk, but thiouracil caused a small but statistically significant decrease. Thyroxine increased markedly the cocarboxylase content of the milk, while a small but statistically significant increase occurred also in the protein-bound thiamine fraction. These increases were mostly at the expense of the free thiamine which was decreased by thyroxine injection. Thiouracil caused the opposite change, a decrease in the cocarboxylase with increase of the free thiamine.

As in human milk, when the phosphatase increased, the cocarboxylase decreased. Similarly, there were close negative correlations between phosphatase and protein-bound thiamine. The correlations between phosphatase and free thiamine were positive. These correlations were found in all cows, irrespective of treatment with thyroxine or thiouracil.

The authors discuss the significance of these changes in phosphatase and cocarboxylase (thiamine pyrophosphate). Thyroxine increases the concentration of enzymes in the body, while thiouracil causes the reverse change. The smaller amount of phosphatase secreted in the milk during thyroxine treatment may denote a retention of the enzyme for synthetic activity in the gland.

TERATOGENIC EFFECTS OF FOLACIN DEFICIENCY

Teratology, the branch of science dealing with abnormal and irregular growths or monstrosities, has been implicated from time to time as being under nutritional influences. In some of the early work with manganese deficiency in the chicken, it was observed that certain fetal abnormalities (parrot beaks and chondrodystrophy) were present at hatching. The hydrocephalic condition in rats noted by L. R. Richardson and A. G. Hogan (*J. Nutrition* **32,** *459 (1946)*) was observed to be under the influence of dietary folacin and vitamin B_{12} (B. L. O'Dell, J. R. Whitley, and Hogan, *Proc. Soc. Exp. Biol. Med.* **76,** *349 (1951)*).

O. B. Ross, P. H. Phillips, G. Bohstedt, and T. J. Cunha noted congenital abnormalities (skeletal anomalies, edema, blindness and paralysis) in swine when the proteins of the maternal diets were vegetable in origin (*J. Animal Sci.* **3,** *406 (1944)*). D. A. Karnofsky, P. A. Patterson, and L. P. Ridgway produced congenital deformities in chick embryos by the injection of folacin antagonists (*Proc. Soc. Exp. Biol. Med.* **71,** *447 (1949)*); similar abnormalities were observed in chicks from folacin-deficient hens by M. L. Sunde, W. W. Cravens, C. A. Elvehjem, and J. G. Halpin (*Poultry Sci.* **29,** *696 (1950)*).

A variety of congenital deformities induced by vitamin deficiencies have been reported by Warkany and associates (J. Warkany and R. C. Nelson, *Anat. Record* **79,** *83 (1941)*; Warkany, Nelson, and E. Schraffenberger, *Am. J. Dis. Child.* **64,** *860 (1942)*; Warkany and Schraffenberger, *Arch. Ophth.* **35,** *150 (1946)*; J. G. Wilson and Warkany, *Am. J. Anat.* **83,** *357 (1948)*).

A detailed study of the teratogenic influence of a specific deficiency of folacin was recently reported by M. M. Nelson, C. W. Asling, and H. M. Evans (*J. Nutrition* **48,** *61 (1952)*). Normal female rats were fed a folacin-deficient diet of the following percentage composition: alcohol-extracted casein, 24; sucrose 62.5; hydrogenated cottonseed oil, 8; salts, 4; succinylsulfathiazole,

1; and a crude folacin antagonist (x-methyl-PGA), 0.5. This diet was fortified with all the vitamins known to be required by the rat, plus ascorbic acid. The female rats were placed on the diet seven to fifteen days after they were pregnant; implantation was presumed to have been established when erythrocytes were found in the vaginal smear.

Fetal resorptions were observed in 100 per cent of cases when feeding of the folacin-deficient diet was begun on the seventh or the ninth day of gestation. Resorption was reduced to 4 per cent when the diet was not fed until the twelfth gestation day onward. Multiple congenital abnormalities were noted in the young when the feeding of the deficient diet was started between the tenth and thirteenth day of gestation. Among these, fetuses examined on the twenty-first day of gestation (day before normal parturition) were found to be alive as judged by a beating heart and reflex movements, but the lungs failed to expand. Ninety-five per cent of such fetuses were found to be markedly abnormal macroscopically and were characterized by marked anemia, edema, and multiple skeletal and visceral abnormalities. The anemia was especially severe, with hemoglobin values as low as 1 to 3 g. per 100 ml. of blood and red blood cell counts of 250,000 to 500,000 per cubic millimeter. Other abnormalities noted were cleft palate, retardation in facial development, syndactylism, and short or curled tails. The eyes also exhibited retarded development and a high incidence of lens fiber degeneration (Morgagnian-type cataract).

When the diet was fed from the eleventh day of gestation onward, the 21 day old fetuses averaged 58 per cent of normal weight. Organs that were most severely affected were kidneys and lungs. These were reduced in size to 10 to 16 per cent, respectively, of their normal weights for 21 day old fetuses.

The timing of the deficiency of folacin had a striking influence on the type of abnormalities present in the fetuses. Whereas practically all the young had all of the above abnormalities when the mothers were placed on the deficient diet on the tenth or eleventh day of gestation, 35 per cent of the young were normal when the feeding of the deficient diet was delayed to the twelfth day. Among the latter, only 2 per cent had cleft palate. Delaying the deficiency one day more (deficient diet from the thirteenth day of gestation) resulted in only 30 per cent of abnormal young, with a slight edema being the principal abnormality. No abnormalities were observed in the young when the deficient diet was fed from the fifteenth day of gestation onward.

The timing of the deficiency was observed to be much more important than the severity of the deficiency. For example, when the diet contained 0.4 per cent of folacin antagonist from the tenth day of gestation onward, the mother consumed on the average 700 mg. of the antagonist during gestation, and all of the fetuses were abnormal. In contrast, when the feeding of the diet containing 0.5 per cent of antagonist was delayed to the twelfth day of gestation, only 65 per cent of the fetuses were abnormal, the total intake of the antagonist during gestation being 720 mg.

In these recent studies, no impairment of reproduction or congenital abnormalities in the fetuses were observed in control animals receiving the same diet supplemented with 50.5 mg. of folacin. The abnormalities observed were therefore apparently due to an uncomplicated folacin deficiency.

Apparently many of the teratogenic effects of vitamin deficiencies are nonspecific and more or less resemble each other regardless of the deficiency. For example, retardation of kidney development, various skeletal abnormalities, edema and eye abnormalities were observed in rat fetuses by Warkany and associates in maternal

deficiencies of vitamin A and riboflavin. What appears to be more important than the nature of the deficiency is the timing. In this respect many of the observed teratogenic effects of vitamin deficiencies appear to have a common property. They appear to exert their marked effects if instituted before the third week of gestation in animals that have a three-week gestation period (rats, chicks).

The importance of diet in gestation as it influences fetal nutrition is particularly emphasized by Nelson and co-workers. The female rats used in the study made normal weight gains of about 100 g. during gestation when the deficient diet was delayed until the twelfth day of gestation and were apparently in good health. In other words, severe fetal abnormalities attributable to a deficiency of folacin were evident in the young when the mothers were apparently in good health.

PATHOGENESIS OF RENAL INJURY IN CHOLINE DEFICIENCY

Young rats fed a diet deficient in choline develop characteristic renal lesions after about one week (*Nutrition Reviews* **1,** *156 (1943)*; **2,** *314 (1944)*; **4,** *345 (1946)*). The kidneys become swollen and discolored, and demonstrate cortical necrosis on histologic examination. Although most animals will die during the early stage of renal damage, a few will apparently recover. Pathologic examination of the kidneys after recovery shows fibrosis of the glomeruli and tubules with foci of calcification.

The pathogenesis of the renal lesion in choline deficiency has been extensively studied. The importance of primary venous stasis with secondary tubular damage has been emphasized by F. I. Desseu and J. J. Oleson (*Proc. Soc. Exp. Biol. Med.* **64,** *278 (1947)*). These investigators were able to prevent renal necrosis by decapsulation. To obtain evidence that the observed pathologic changes had a neurovascular basis, they treated immature rats fed a choline-deficient diet with atropine sulfate. Of 21 rats so treated, 10 appeared to have suffered no renal lesions.

In contrast, the importance of primary tubular damage with secondary vascular complications was suggested by the thorough histopathologic studies of W. S. Hartroft (*Brit. J. Exp. Path.* **29,** *483 (1948)*). The earliest renal lesion found was the appearance of fat droplets in the tubular cells. Soon thereafter generalized tubular swelling occurred which appeared to compress the cortical capillary plexus, resulting in tubular ischemia, necrosis and hemorrhage. Although Hartroft emphasized the importance of increased intrarenal tissue pressure in producing necrosis, he was unable to alter the development of the lesion by renal decapsulation. No protective effect of atropine could be demonstrated.

The importance of intrarenal pressure in the development of the renal lesion of choline deficiency has been reinvestigated by J. H. Baxter (*J. Exp. Med.* **96,** *401 (1952)*). Male rats weighing 30 to 40 g. were fed a choline-deficient diet. Control rats became ill after four to seven days of the diet and by twelve days only 17 per cent of the rats were still alive. Rats, the left kidney of which was decapsulated either by the abdominal or lumbar route prior to feeding a choline-deficient diet, appeared sick at the expected time of four to seven days. However, about two thirds of the animals survived the period of acute illness, a substantial reduction of mortality. Histologic damage in the decapsulated kidney was compared with the intact right kidney. Occasionally the decapsulated kidney appeared entirely normal. More frequently some degree of damage could be demonstrated in the kidney operated upon, but it was always less than the control right

kidney. At later stages when the edema and congestion had subsided the decapsulated kidney was larger and firmer and retained a well-defined cortex as compared to its unoperated mate.

When the decapsulation was performed on the third day of the deficient diet some protection was afforded (46 per cent survival). However, when the operation was delayed until the fourth or fifth day the mortality was not significantly different from the control group.

An attempt was made to distinguish between the beneficial effect of renal decapsulation due to the relief of pressure and that due to the establishment of collateral circulation. This was done by enclosing the decapsulated kidney in loose plastic or rubber envelopes. Because of technical difficulties the isolation of the kidney was not uniform. It did seem likely, nevertheless, that the development of adhesions to surrounding tissues was not necessary to afford protection. Because renal denervation or atropinization did not protect young rats against renal damage when fed low choline diets, the author considered nervous factors as not important in the pathogenesis of the lesion.

In summary, the rapidly growing immature rat is unable to synthesize sufficient choline for phospholipid production. This leads to tissue damage by unknown mechanisms in the liver and kidney. The kidney lesions begin as deposition of lipid droplets and progress to cellular edema and tubular damage. The extent of the lesion is largely determined by the extent of compression of renal capillaries and veins. Ischemia of the kidney with necrosis is the result of increased intrarenal pressure and can be partially prevented by decapsulation. The evidence favoring a primary vasomotor disturbance in choline deficiency is not convincing. The ability to synthesize choline eventually becomes adequate for the metabolic requirement, and the lesions regress and continued existence is possible on the low choline diet.

METHIONINE AND CYSTINE IN WOUND HEALING IN ALBINO RATS

It has been known for centuries that certain poorly nourished patients are subject to delay in, or failure of, healing of wounds. Since wound repair implies cellular proliferation which in turn requires protein as a principal building constituent, it is to be expected that the relationship of protein metabolism to wound healing would be extensively investigated. Prolongation of the lag period, delay in epithelialization and in proliferation and maturation of connective tissue, and increase in infection, morbidity, and mortality have been observed in protein-deficient animals and patients. However, relatively little information on the importance of specific amino acids is available.

Supplementation of a low protein diet by tryptophan, valine and lysine had no apparent effect on the healing of open wounds in rats (H. P. Morris, C. S. Dubuik, and T. B. Dunn, *J. Nat. Cancer Inst.* **5,** *271 (1944/45)*). However, the authors felt that the number of animals studied was too small to attach any significant weight to these observations. S. A. Localio, M. E. Morgan, and J. W. Hinton (*Surg. Gynec. Obstet.* **86,** *582 (1948)*; *Nutrition Reviews* **6,** *274 (1948)*) reported a shift of the curve of wound healing towards normal in protein-deficient rats given a daily supplement of 150 mg. of DL-methionine subcutaneously. As an explanation for the effect of methionine, Localio and associates suggested that the sulfhydryl radical is deficient and not readily available to the wound of the protein-deficient rat, and deficient –SH enzyme activity may be one of the reasons for

delayed healing. It was postulated by these authors that methionine would furnish the necessary –SH groups. Further investigations of the relationship of nitrogen and sulfur metabolism to the healing of experimental wounds have been recently reported by Williamson and his co-workers.

In the first study (M. B. Williamson, T. H. McCarthy, and H. J. Fromm, *Proc. Soc. Exp. Biol. Med.* **77,** *302 (1951)*), four groups of 24 young adult female albino rats weighing about 175 g. were used. The animals in group I were fed a basal diet of 20 per cent casein supplying 2.56 g. nitrogen per 100 g. of diet; those in group II, a diet of 6 per cent casein supplying 0.76 g. nitrogen per 100 g. of diet; group III, a diet of 6 per cent casein and 11.3 per cent gelatin, supplying 2.55 g. nitrogen per 100 g. of diet; and group IV, the diet of group III plus a supplement of 0.147 per cent methionine. All the diets were isocaloric and contained the same amounts of vitamin supplements. Fifteen g. of each diet were offered each rat daily. On the average, about two thirds of the diet offered was ingested. Distilled water was given ad libitum to all animals.

After a four-day period of adjustment to the various diets, a skin incision 2 cm. in length was made in each animal in the middorsal region under nembutal and ether anesthesia. The wounds were closed with Michel clips. At approximately weekly intervals, 8 rats from each group were killed and the tensile strength of a 0.5 cm. section of the healing wound determined. The mean daily caloric intake for the twenty-one days after wounding was, on the average, about the same in the animals fed the 6 per cent or 20 per cent casein diets. The nitrogen intake was perforce approximately three times greater in the 20 per cent casein group, an average of 266 mg. nitrogen daily as compared with 78 mg. nitrogen daily in the 6 per cent casein groups. In keeping with previous findings, the rate of healing of the wounds in the latter group was, on the average, slower than in the 20 per cent casein group.

A similar delay in wound healing was observed in the group of animals receiving the unsupplemented 6 per cent casein and 11.3 per cent gelatin diet, although the average daily nitrogen and caloric intake of this group was similar to that of the 20 per cent casein group. The observed difference in wound healing is presumably a reflection of the low biologic value of gelatin. When methionine was added to the casein-gelatin diet there was no increase in nitrogen intake, but the rate of wound healing was increased to about the rate of the 20 per cent casein group.

It is evident that there is no correlation between total nitrogen intake and rate of healing among the four groups of animals studied by Williamson and his associates, nor is there a definite correlation between nitrogen retention and wound healing. The mean daily nitrogen retention for the twenty-one days after wounding was, on the average, 78 mg. nitrogen in the 20 per cent casein group, 10 mg. nitrogen in the 6 per cent casein group, 47 mg. nitrogen in the unsupplemented casein-gelatin group, and 56 mg. nitrogen in the methionine-supplemented casein-gelatin group. However, suggestive correlations among sulfur intake, sulfur balance and wound healing were observed. The higher the sulfur intake, the higher the observed sulfur balance, and the higher the rate of wound healing. In the 20 per cent casein group the mean daily sulfur intake during the wound healing period was, on the average, 13.2 mg. while the daily balance was +11.8 mg.; in the 6 per cent casein group, 3.6 mg. and +2.6 mg. respectively; in the unsupplemented casein-gelatin group, 8.2 mg. and +6.4 mg. respectively; and in the methionine-supplemented casein-gelatin group, 23.1 mg. and +14 mg. respectively.

In a subsequent study, M. B. Williamson and H. J. Fromm (*Proc. Soc. Exp. Biol. Med.* **80,** *623 (1952)*) observed that sulfur

retention and rate of wound healing in rats receiving a 6 per cent casein basal diet were affected by cystine supplementation to the same extent as by an equivalent amount of methionine on the basis of the sulfur content of the two compounds. Alanine was ineffective. Supplemental cystine and methionine were also equally effective in promoting wound healing when given in comparable amounts (on the basis of sulfur content) to animals on a basal diet in which all casein was replaced by alanine. This indicated that the effect of cystine was not due to a sparing by cystine of the methionine available in casein. A correlation between nitrogen retention and wound healing, as well as between sulfur retention and wound healing was observed. Williamson and Fromm feel that methionine is chiefly converted to cystine before being used in the healing process.

To investigate the mode of action of methionine and cystine, three groups of rats were fed a 5 per cent casein diet supplemented with 100 mg. of ethionine. One group received no further supplement, the other two groups received 100 mg. of either cystine or methionine sulfur per 100 g. of diet. The latter two groups had slower healing rates than the control animals which received no methionine or cystine. These data are interpreted by Williamson and Fromm to indicate interference with the utilization of both cystine and methionine by ethionine. They feel further that since a low healing rate was observed in the cystine supplemented group despite the administration of large amounts of cystine, the cystine requirement was no longer the limiting factor in the healing process and that ethionine interfered with the utilization of methionine for purposes other than cystine formation. They conclude that methionine is *also* required for wound healing and that the methionine requirement during wound healing is primarily for protein synthesis, whereas the cystine required may be used, to some extent, for reactions other than protein synthesis. It should be pointed out that these conclusions are highly speculative and not specifically indicated by the data presented by the authors.

It would appear that the sulfur-containing amino acids are important in wound healing in rats. Other amino acids may be equally important. Further studies are required to elucidate the mechanisms of these effects and the interrelationships of the amino acids among themselves and other nutrients in regard to wound healing. Studies in other species, including human beings, are also needed.

NOTES

Letter to the Editor

Dear Sir:

It is perplexing and a matter of serious concern that, in this day of high-speed communication, your writers should not have availed themselves of the opportunity to check their statements so as to avoid being guilty of the gross misrepresentations regarding the lactose supplements (we eschew the word "placebo") contained in your review of our Shaker Heights Nutrition Study (*Nutrition Reviews* **11,** *42 (1953)*).

Let me assure you that knowledge as to what type of supplement any child receives is one of the most carefully guarded secrets within the jurisdiction of the Medical and Health Departments.

This means, specifically, that no teacher, parent, nurse, physician, clerk, technical assistant, the subjects themselves, nor even I, knew the exact nature or composition of any supplement at any given time without recourse to carefully preserved records, access to which is limited to qualified personnel. Indeed, our employment of preparations of "various sizes, shapes and colors" as described in our paper, not only prevented anyone from knowing improperly "who was getting what" and hence, prejudice or bias from such a source, but it also provided each subject with an equal chance of believing the supplements to be beneficial, if he were disposed to do so at all.

Among many other points deserving sharp rejoinders I note but two: First, your writers could also have learned—by inquiry prior to publishing your review—that the 4 unsupplemented children in Group D, *i.e.*, the "withdrawals" from Group C, were thus classified for the reason that their parents decided, during the first week or so, to try measures of their own, and requested releases. We welcomed this opportunity at once, for, as a result of *their* choice, *not ours*, we were afforded a most desirable group of "negative controls"—the value and importance of which has been entirely overlooked by, and lost upon, your writers. Second, one cannot help wondering how or why, in their 1690-word article, the evidence of two independent responses to vitamin B_{12}, clearly revealed in our Figure 3, was so completely and utterly ignored by your reviewers.

Needless to add, we shall continue to employ the Grid Technique in the School Health Services; we shall advise that others do likewise—not only as a quality control chart on child growth as it was originally intended to be used, but also as an aid in the routine of School Health, for the benefit of all concerned, and I, myself, shall continue, as a direct result of our studies, though also from observations I have made in private practice, to recommend vitamin B_{12} (among other things) in the management of Simple Growth Failure in School Children.

HOWARD H. HOPWOOD, M.D., *Director*
Medical Services,
Shaker Heights Public Schools,
Shaker Heights, Ohio

Editor's Note: Reviews must be written on the basis of the available literature and not on the basis of additional information obtained by correspondence. We also think growth charts of the Grid type are helpful tools in the appraisal of health and have a useful role in school health services. The review questioned by Dr. Hopwood represented the combined work of four members of the editorial staff and three outside consultants, physicians recognized for their research in growth and development in children.

CLINICAL NUTRITION BRIEFS

"Failure to wean the baby from milk at the proper time may result in refusal of other food and subsequent development of a relatively persistent anemia. The second most important feature was probably the failure to continue the administration of supplementary vitamins beyond the first few months of life." (*Nutrition Reviews* **11,** *3* (*1953*), Malnutrition in Young Children.)

"Adequate nutrition is possible in diets that are made up of quite different combinations of foods, and even in the United States there is considerable variation in the dietary pattern between regional population groups." (*Nutrition Reviews* **11,** *8* (*1953*), Diet During Pregnancy.)

"Nearly all of the children with pellagra showed some evidence of lung involvement. Radiologically the lesions are similar to and have often been reported as tuberculosis. The authors were unable to demonstrate tuberculosis in most of the cases by means of the Mantoux test, sedimentation rates, and examinations of gastric washings for the Koch bacillus. Fever is generally lacking and the lesions regress slowly with diet therapy." (*Nutrition Reviews* **11,** *36* (*1953*), Infantile Pellagra.)

"Patients with Cushing's syndrome demonstrated a marked increase in the percentage of isotopic nitrogen excreted within these forty-eight hours as compared to the normal individuals, but usually not with the appearance of negative nitrogen balance." (*Nutrition Reviews* **11,** *38* (*1953*), Nitrogen Metabolism in Cushing's Syndrome.)

"Iron which is given intravenously is retained almost quantitatively by the body. Large amounts given to animals have caused no structural changes in the tissues; however, excessive deposits of iron during the years may produce damage to the tissue. Care is necessary in the intravenous administration of iron." (*Nutrition Reviews* **11,** *47* (*1953*), The Use of Intravenous Iron.)

"Treatment with niacinamide cleared the diarrhea within one day, while the mucous membrane lesions required a week, and the glossal papillae returned to normal only after a month. Dermatitis began to improve within forty-eight hours and the skin was practically normal at the end of a month." (*Nutrition Reviews* **11,** *70* (*1953*), Requirements for Niacin and Tryptophan in Man.)

"The mechanism by which the low fat, low cholesterol diet induced such a rapid fall in serum cholesterol is not clear." (*Nutrition Reviews* **11,** *72* (*1953*), Dietary Treatment of Essential Familial Xanthomatosis.)

"The results of these experiments provide important evidence of an abnormality in the metabolism of vitamin B_6 in pregnancy with increased requirement for this vitamin." (*Nutrition Reviews* **11,** *75* (*1953*), Enhanced Requirement for Vitamin B_6 in Pregnancy.)